# When the Leader Gets Loose

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Supporting Information



# When the Leader Gets Loose: In Vivo Biosynthesis of a Leaderless Prenisin Is Stimulated by a *trans*-Acting Leader Peptide

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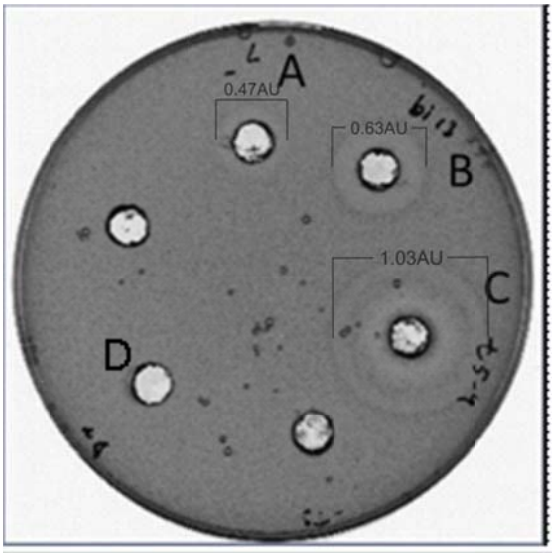


**Figure S1**. Antimicrobial activity assay of the Ni-NTA purified nisin variants. Nisin variants modified by NisBTC enzymes in vivo were purified and applied to the agar plate containing nisin-sensitive indicator strain *L. lactis* NZ9000 expressing NisPT. A) nisin variant NisA(24-57)-H6 maturated without the leader was applied. B) The purified sample from the bicistronic system that expressed the leader and the propeptide part *in trans*, were applied. C) wild-type prenisin, used as a positive control. D) elution buffer with NisP was applied (negative control). A halo indicates the presence of active nisin processed by NisP to remove the nisin leader. AU: arbitrary units. The squared values of inhibition zone radii are proportional to ln(concentration).

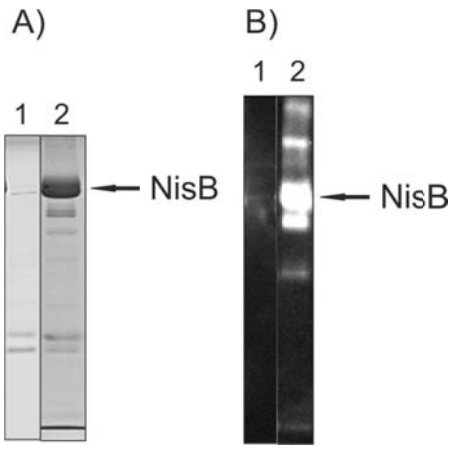


**Figure S2**. Elutions from Ni-NTA purification of His-tagged prenisin Lane 1: Strain containing wild type pIL3BTC vector and a leaderless NisA(24-57)-H6; lane 2: strain containing wild type pIL3BTC vector and NisA-H6; S2A. SDS-PAGE analysis of NisB co-purified with C-terminally extended His-tagged prenisin NisA-H6 S2B. Western blot of NisB co-purified with C-terminally extended His-tagged prenisin NisA-H6 detected by anti-NisB antibodies.

Primers used in this study:

pNZnisA-H6 vector,[10] encoding for NisA-H6, was used as a template. The following set of primers was used to divide the *nisA* gene into two parts, for preparing the construct for the *in trans* expression of the nisin leader and the propeptide part.

R_nisA_leader_bicistr: 5'-TCAGC GTGGT GATGC ACCTG AATC-3'

F_nisA_leader_bicistr: 5'-ACATG ATCAA TTATA AGGAG GCACT CACCA TGATT ACAAG TATTT CGCTA TGTAC ACCC-3'

In frame deletion of the region encoding for the nisin leader was made using the following set of primers.

F_del_leader_nisA: 5'-ATGAT TACAA GTATT TCGCT ATGTA CAC-3'

R_del_leader_nisA: 5'-GGTGA GTGCC TCCTT ATAAT-3'

**Table S1**. Dehydration of nisin variants containing a C-terminal extension GSIEGR with a His-tag, modified in vivo by NisBTC in *L. lactis,* isolated by Ni-NTA purification and analysed by MALDI-TOF mass spectrometry.

| Mass $[M + H]^+$ w/o Met1 [Da] | | | |
|---|---|---|---|
| Mutant | Dehydration extent | Observed [Da] | Calculated [Da] |
| NisA(24-57)-H6 | 0 + Met | 5046.34 | 5048 |
| | 1 + Met | 5028.46 | 5030 |
| | 2 + Met | 5010.51 | 5012 |
| | 3 + Met | 4993.32 | 4994 |
| | 4 + Met | 4975.31 | 4976 |
| | 5 + Met | 4957.41 | 4958 |
| | 4 – Met | 4844.51 | 4845 |
| | 3 – Met | 4862.48 | 4863 |
| NisA(1-23) and NisA (24-57)-H6 (*in trans*) | 5 – Met | 4826.42 | 4827 |
| wild-type NisAH6 | 9 – Met | 7109.79 | 7110 |
| | 8 – Met | 7091.67 | 7092 |